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Testing mechanisms of N-enrichment-induced species loss in a semiarid Inner Mongolia grassland: critical thresholds and implications for long-term ecosystem responses

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The increase in nutrient availability as a consequence of elevated nitrogen (N) deposition is an important component of global environmental change. This is likely to substantially affect the functioning and provisioning of ecosystem services by drylands, where water and N are often limited. We tested mechanisms of chronic N-enrichment-induced plant species loss in a 10-year field experiment with six levels of N addition rate. Our findings on a semi-arid grassland in Inner Mongolia demonstrated that: (i) species richness (SR) declined by 16 per cent even at low levels of additional N (1.75 g N m⁻² yr⁻¹), and 50–70% species were excluded from plots which received high N input (10.5–28 g N m⁻² yr⁻¹); (ii) the responses of SR and above-ground biomass (AGB) to N were greater in wet years than dry years; (iii) N addition increased the inter-annual variations in AGB, reduced the drought resistance of production and hence diminished ecosystem stability; (iv) the critical threshold for chronic N-enrichment-induced reduction in SR differed between common and rare species, and increased over the time of the experiment owing to the loss of the more sensitive species. These results clearly indicate that both abundance and functional trait-based mechanisms operate simultaneously on N-induced species loss. The low initial abundance and low above-ground competitive ability may be attributable to the loss of rare species. However, shift from below-ground competition to above-ground competition and recruitment limitation are likely to be the key mechanisms for the loss of abundant species, with soil acidification being less important. Our results have important implications for understanding the impacts of N deposition and global climatic change (e.g. change in precipitation regimes) on biodiversity and ecosystem services of the Inner Mongolian grassland and beyond.

Keywords: chronic nitrogen enrichment; above-ground competition; recruitment limitation; soil acidification; stability; drought resistance

1. INTRODUCTION

Nitrogen (N) is one of the most limiting nutrient elements controlling plant growth, reproduction, species composition and primary production in many terrestrial ecosystems [1–3]. Global nitrogen availability, which has doubled globally over the last 50 years through industrial and agricultural processes, is one of the major drivers of widespread biodiversity loss and degradation of ecosystem services worldwide [4–6]. Experimental studies in grasslands have demonstrated N addition consistently decreases species richness (SR) and promotes a few species with particular traits that enable them to exploit the additional N [7–10]. Such reductions in SR and shifts in functional groups composition can have profound impacts on multiple ecosystem functions, such as N retention, carbon sequestration, above- and below-ground biomass, and stability [11–14]. Thus, a better understanding of how changes in biodiversity and ecosystem services following N enrichment are affected by key abiotic and biotic factors is fundamental to management and preservation of biodiversity in the light of global change [4,15,16].

Several hypotheses regarding N-enrichment-induced species loss are focused mainly on the direct effects of N accumulation and indirect effects of soil mediated acidification on plant growth [10,17]. N addition increases primary production and hence causes cascading effects on competitive exclusion including...
assemblage thinning [7,18], the relative importance of above-ground or light competition over below-ground competition [19,20] and reduction of species recruitment [21,22]. For some systems, N addition increases soil acidity and results in toxicity through mobilization of metals, leaching of base cations and changing the balance between nitrogenous compounds [23–25]. After decades of efforts, however, there are few agreements but, rather, results are case-dependent [4,10,17] or even opposite of expectations [26]. This is probably because plant community-level responses to N addition depend on environmental conditions, production response, community composition, species’ relative abundance (RA) and traits of individual species, indicating the importance of species-level responses [9,27]. Compared with the relatively extensive research at the plant community level, only a few studies have evaluated the species-level responses using functional trait-based approach [7,28].

Temporal scale is an important dimension for understanding plant community response to N addition because of the cumulative and lagged effects of N inputs [17,29]. This suggests that there exist ecosystem-specific critical thresholds for N enrichment, below which N might be unavailable to plants as microbial and abiotic processes outcompete plants for excess N [8,30,31]. For example, Bai et al. [8] found that the reduction in SR occurred after three years of N addition at both mature and degraded communities in the semi-arid Inner Mongolia steppe, with the critical threshold for N-induced species loss being around 1.75 g N m⁻² yr⁻¹. After 20 years of N enrichment in the North American grasslands, SR decreased even at the rate of 1 g N m⁻² yr⁻¹, which was previously suggested to be safe to community diversity [32]. Another long-term study in the semi-natural central European grassland found that SR initially declined but began to recover after 25 years of continued N enrichment [33].

In arid and semi-arid ecosystems, plant diversity and primary production are co-limited by water and N [34–36], and thus the responses of plant community to N addition are probably mediated by water availability [37]. Plant responses to N addition were greater in more mesic than drier ecosystems [38], and for a given grassland above-ground biomass (AGB) increase was much larger in wet than dry years [34,35]. According to hypotheses concerning N-induced species loss, high water availability could elevate the rate of species loss because of greater production increase and thus more light limitation. A recent study, however, showed that fertilization diminished plant diversity when light was not limiting [26]. Harpole et al. [39] proposed that water addition promoted the production in response to N addition, but had no effect on SR response. Thus, it remains unclear to what extent water and light availability affect SR response to N addition.

In this study, we present results from a 10-year field experiment with six levels of N addition rate at a semi-arid Inner Mongolia grassland [8], which is part of widely distributed grassland across the Eurasian Steppe region [35]. Our study addresses the following three questions: first, how do the patterns and critical thresholds for N-induced plant species loss vary across different levels of organization (i.e. plant species, functional group and community) and with experimental duration? Second, how does water availability (i.e. wet versus dry years) affect the responses of SR and primary production to N addition? Third, what are the major mechanisms controlling chronic N enrichment-induced losses of plant species and changes in ecosystem functioning in terms of biomass production, stability and drought resistance? To address the first and second questions, we used several statistical approaches for assessing long-term changes in species diversity and ecosystem functioning at the levels of plant species, functional group and the whole community resulting from N enrichment. To address the third question, using functional trait-based approach, we tested six interrelated hypotheses which have been proposed to be the major mechanisms driving the N enrichment-induced changes in plant species diversity and ecosystem functioning [7,19,22,23,29,35]. These hypotheses include the random loss, shift from below-ground competition to above-ground or light competition, recruitment limitation, N addition duration, water and N co-limitation, and soil acidification.

2. MATERIAL AND METHODS

(a) Study site

The study was conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS; 116°42′E, 43°38′N and 1250 m a.s.l.), which is located in the Xilin River Basin, Inner Mongolia Autonomous Region of China [8]. The mean annual temperature in the study area is 0.3°C, with mean monthly temperature ranging from −21.6°C in January to 19.0°C in July. The mean annual precipitation is 346.1 mm, with about 80 per cent occurring in the growing season from May to September. The field experiment was carried out on a natural steppe community, which has been fenced against grazing by large animals since 1979. The soil is a dark chestnut (Calcic Chernozem according to ISSS Working Group RB, 1998) or loamy sand in terms of texture [8]. Soil pH value is 7.5 and cation exchange capacity (CEC) is 15.7 cmol kg⁻¹. Soil organic carbon, total nitrogen and total phosphorous contents at the top 20 cm layer are 15.0, 1.7 and 0.3 g kg⁻¹, respectively. No fertilizer had been used before this experiment. Before beginning the experiment, the plant community was dominated by Leymus chinensis (Trin.) Tzvel., a widely distributed perennial C₃ rhizomatous grass in the Eurasian steppe region [8,35].

(b) N addition experiment

To explore the effects of N on plant species diversity and ecosystem functioning (i.e. biomass production, stability and drought resistance), we established an N addition experiment in the autumn of 1999. The N addition experiment has been maintained since 2000. There were seven treatments and nine replicates for each treatment, a total number of 63 plots. The plots, each 5 × 5 m in size, were arranged in a randomized block design and separated by 1 m buffers. Each block included a control (no nutrient

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addition) and six levels of N addition rate, i.e. 0, 1.75, 5.25, 10.50, 17.5, and 28.0 g N m$^{-2}$ yr$^{-1}$. Nitrogen was added as commercial pelletized NH$_4$NO$_3$ fertilizer. To ensure that N was the only limiting nutrient [8,40], for all treatments except the control, we also added the same amount of P (10 g P$_2$O$_5$ m$^{-2}$ yr$^{-1}$), S (0.2 mg m$^{-2}$ yr$^{-1}$) and trace elements (Zn, 190 mg m$^{-2}$ yr$^{-1}$; Mn, 160 mg m$^{-2}$ yr$^{-1}$ and B, 31 mg m$^{-2}$ yr$^{-1}$) based on the soil census data [41]. Each year, nutrients were uniformly applied to each N-treatment plot with manual broadcasting in the mid-growing season (1–5 July), coinciding with high temperature and precipitation.

(c) Soil acidification experiment
To assess the relative importance of N input versus soil acidification in influencing SR, we established an additional soil acidification experiment near the N addition experimental site within the same plant community in 2009. There were eight treatments and five replicates for each treatment. A total of 40 plots, each of 2×2 m, were laid out in a randomized block design and separated by 1 m walkways. Each plot was fenced with a 25 cm height iron sheet, of which 20 cm was driven into soil. The treatments include a control (nothing added) and seven levels of acid addition rate (i.e. 0, 2.76, 5.52, 8.28, 11.04, 13.80 and 16.56 mol H$^+$ m$^{-2}$) in the form of sulphuric acid solution. Acid addition was carried out in September 2009, June 2010 and September 2010, respectively. For each time, one dose of 98 per cent acid was sprayed. Acid addition was carried out in 2009, June 2010 and September 2010, respectively. For each time, one dose of 98 per cent acid was sprayed. For each treatment, the dry mass of plants and soil organisms, each plot received an additional 30 and 50 l well water, respectively, before and after the acid solution was sprayed.

(d) Filed sampling and measurements
For the N addition experiment, above-ground vegetation was sampled each year between 25 August and 2 September by clipping all plants at the soil surface using a 0.5×1 m quadrat, which was randomly placed in each plot without spatial overlap of quadrats among all years [8]. To avoid edge effects, the quadrat was placed at least 50 cm inside each plot. For each species, the plant height and number of individuals were measured before clipping. Living vascular plants were sorted into species, and all plant materials, including litter and standing dead, were oven-dried at 65°C for 48 h and weighed. For each treatment, the dry mass of all living plants and number of species per quadrat were averaged over the nine replicates to estimate the total AGB and SR [8]. For the soil acidification experiment, plant height, density and number of species were measured during 28–30 August in 2010 and 2011 using a 0.5×0.5 m quadrat, which was located in the northeast part of each 2×2 m plot.

For the N addition experiment, soil samples were taken from the surface to a depth of 20 cm in 7–18 July, 2009. Within each plot, one soil core was randomly collected using a 3-cm diameter soil auger. Soil cores were sieved through a 2-mm mesh and air-dried. The soil pH values were measured in water suspension (water : soil = 2.5 : 1) by a pH metre (FE20—FiveEasy). For the soil acidification experiment, soil samples were collected on 25 August in 2010 and 2011, and soil pH values were determined using the same method.

(e) Plant functional traits
To test the relative importance of abundance versus functional trait-based mechanisms underlying the chronic N enrichment-induced species loss, we quantified 17 plant functional traits for all species in the experiment (see the electronic supplementary material, table S1). These traits, including mass- and area-based leaf N contents, leaf C : N, specific leaf area, plant height, stem : leaf biomass ratio, root : shoot biomass ratio, net photosynthetic rate, instantaneous water use efficiency, specific root length of the first-, second- and third-order roots, reproductive allocation (fruit to total plant biomass ratio), and seed size and grain mass, were measured using standard methods [42]. Foliar $\delta^{13}$C values, leaf nitrogen resorption efficiency, and leaf nitrogen use efficiency were obtained from published data (see electronic supplementary material, table S1). Data of plant life history, clonality and life form for different species were obtained from the Flora of Inner Mongolia. Trait plasticity index was calculated as in Godoy et al. [43].

(f) Species abundance class and response variables
To facilitate our analysis and interpretations, based on average RA of each species in control plots during 2005–2009, plant species were classified into two abundance classes, i.e. common species (RA > 1%) and rare species (RA < 1%) [32]. To allow direct comparison across time and between N addition and soil acidification experiments, the SR response ratio was calculated as the ratio of the mean SR in each N treatment to the mean SR in control (i.e. $\text{SR}_{\text{treat}}$/SR$_{\text{control}}$). Similarly, the AGB response ratio was calculated as the ratio of the mean AGB in each N treatment to the mean AGB in control (i.e. $\text{AGB}_{\text{treat}}$/AGB$_{\text{control}}$).

To evaluate the effect of chronic N enrichment on stability of production, we used the coefficient of variation of AGB and drought resistance as indices of stability. The coefficient of variation of AGB over three replicates of each treatment was used as the measure of intra-annual variance of AGB (intra-annual $\text{CV}_{\text{AGB}}$). The inter-annual variance of AGB (inter-annual $\text{CV}_{\text{AGB}}$) was calculated as the coefficient of variation of AGB of each plot over five years (2005–2009). For each treatment, the drought resistance was expressed as the ratio of the AGB in dry year to the AGB in normal year [44], i.e. $\text{AGB}_{\text{dry}}$/$\text{AGB}_{\text{normal}}$ in this study.

(g) Statistical analyses
Statistical analyses were performed using SAS v. 8.0 (SAS Institute, Cary, NC, USA, 2001). Repeated measures analyses for SR and AGB were performed with mixed linear model (Proc Mixed) using N treatment, year and their interactions as fixed effects. ANOVAs followed by a Duncan’s multiple-range test
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Table 1. Repeated measures analyses with mixed linear model (PROC MIXED) for species richness and above-ground biomass using N treatment (N), year (Y) and their interactions as fixed effects.

<table>
<thead>
<tr>
<th>response</th>
<th>term</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>all species</td>
<td>N</td>
<td>41.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>41.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N x Y</td>
<td>1.13</td>
<td>0.3129</td>
</tr>
<tr>
<td>common species</td>
<td>N</td>
<td>26.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>58.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N x Y</td>
<td>2.34</td>
<td>0.0008</td>
</tr>
<tr>
<td>rare species</td>
<td>N</td>
<td>28.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>19.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N x Y</td>
<td>2.53</td>
<td>0.0003</td>
</tr>
<tr>
<td>above-ground biomass</td>
<td>N</td>
<td>25.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>43.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N x Y</td>
<td>2.92</td>
<td>&lt;0.0001</td>
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were performed to test for differences between controls and each N-treatment level each year. Regression models were used to determine the relationship between N addition rate and various responses (adjustments for non-linearity were made as needed). We used linear regressions to examine relationships between responses of SR and biomass and experimental duration and June–July precipitation. The critical threshold ($N_{crit}$) was estimated by extrapolating the fitted curve and its 95% confidence interval (CI) [32].

3. RESULTS

(a) Response of species richness

Repeated measures analysis with PROC MIXED for SR, using N treatment (N), year (Y) and their interactions as fixed effects, showed that the effects of N and experimental duration were highly significant for total SR and two abundance classes, i.e. common species and rare species (table 1). The interactions of N x Y were significant for both the common and rare species (table 1). Our analysis further revealed that the effects of N treatment, year and N x Y on SR response ratio were all significant for both the common and rare species (see electronic supplementary material table S2). Total SR declined with increasing N addition rate throughout the 5-year period (figure 1a). The slope of the SR–N relationship decreased linearly with increasing N addition duration ($R^2 = 0.70, p < 0.05$; data not shown). By 2009 (the 10th year), nine species (approx. 75%), on average, were excluded from plots at the two highest N addition rates (17.5 and 28 g N m$^{-2}$ yr$^{-1}$), compared with those of control plots in 2005 and 2006 (figure 1a). Patterns of SR response to N were comparable between the common and rare species (figure 1b,c).

No significant difference in SR was found between the control (no nutrients added) and the zero-N treatment (added P, S and trace elements but not N) (For Treatment, F = 0.14, p = 0.7054; for time x treatment, F = 1.36, p = 0.2557).

SR response ratio showed a log-linear function of N addition rate; and SR started to decline at the addition rate of 1.75 g N m$^{-2}$ yr$^{-1}$ throughout four of the five years, saturating at 17.5 g N m$^{-2}$ yr$^{-1}$ (figure 2a).

Total SR decreased by 15 per cent relative to control even at the lowest addition rate of 1.75 g N m$^{-2}$ yr$^{-1}$. The predicted critical threshold ($N_{crit}$) was 0.85 g N m$^{-2}$ yr$^{-1}$ with a 95% CI of 0.44–1.51 g N m$^{-2}$ yr$^{-1}$.

For rare species, a similar negative relationship between SR response and N addition rate was observed, with richness reduction occurring at the addition rate of 1.75 g N m$^{-2}$ yr$^{-1}$ (figure 2c). The slope of the fitted curve for rare species was significantly steeper (slope $= -0.26$) than that for total species (slope $= -0.15, p < 0.01$), and it was greater at lower N addition rates. The $N_{crit}$ for rare species was 0.22 g N m$^{-2}$ yr$^{-1}$, with a 95% CI of 0.09–0.50 g N m$^{-2}$ yr$^{-1}$. For common species, in contrast, the reduction in SR increased linearly with N addition rate, and SR began to decline mostly at the 10.5 g N m$^{-2}$ yr$^{-1}$ rate, saturating at 17.5 g N m$^{-2}$ yr$^{-1}$ (figure 2b). The $N_{crit}$ for common...
species and (28.0 g N m\(^{-2}\) yr\(^{-1}\)) for both common and rare species, the reduction in AGB was significantly affected by N treatment, experimental duration and their interactions (table 1). For both common and rare species, the reduction in soil pH, however, greater reduction in SR tended to either increase or decline at the addition rate of 5.25 g N m\(^{-2}\) yr\(^{-1}\), compared with control plots (see the electronic supplementary material, figure S1). For example, AGB increased by 87 per cent in 2008 (a wet year; June–July precipitation = 170.7 mm) and only by 32 per cent in 2009 (an extremely dry year, June–July precipitation = 93.7 mm) at the additional rate of 10.5 g N m\(^{-2}\) yr\(^{-1}\), compared with control plots (see the electronic supplementary material, figure S1).

The coefficients of intra-annual variation in AGB did not show significant response to N treatment (figure 3a). The coefficients of inter-annual variation in AGB at first increased with N addition rate, peaked approximately at the addition rate of 17.5 g N m\(^{-2}\) yr\(^{-1}\), and then declined at the highest N addition rate (figure 3b). For a given treatment, the magnitude of inter-annual variation in AGB was generally greater than the intra-annual variation (variation among replicates). The drought resistance declined with increasing N addition rate (figure 3c).

(c) Plant species responses

At the beginning of the experiment, there were 48 vascular plant species and 80 per cent of them were rare species (RA < 1%). The loss of rare species continued throughout five years of the experiment (the 6–10th year; figure 1c). At species level, AGB of rare species varied among N treatments and over time, owing mainly to their low initial abundance (data not shown).

For common species, both the patterns and magnitude of AGB response to N treatment differed substantially among species (see electronic supplementary material, figure S2). The critical thresholds for N addition ranged from 1.75 (Carex korshinskyi) to 10.5 g N m\(^{-2}\) yr\(^{-1}\) (e.g. Leymus chinenis, Achmatherum sibiricum and Agropyron cristatum; electronic supplementary material, figure S2). For Stipa grandis, Chenopodium glaucum and Axyris amaranthoides, AGB tended to either increase or decline at the addition rate of 5.25 g N m\(^{-2}\) yr\(^{-1}\) (see electronic supplementary material, figure S2).

(d) Relationships of species richness with soil pH and above-ground biomass

For both N addition and soil acidification experiments, plant SR declined with reduction in soil pH, which is expressed as pH\(_{\text{control}} - pH_{\text{treat}}\) of the top 20 cm of soil (Δ soil pH, figure 4a). For a given reduction in soil pH, however, greater reduction in SR was found in the N-treatment plots than in the acid-treatment plots. In the N addition experiment, SR tended to decline even with relatively small reduction in soil pH (e.g. Δ soil pH = 0.4). In the

**Figure 2. Relationships between species richness response ratio and N addition rate for (a) all species, (b) common species and (c) rare species over five years.** Species richness response ratio was the average of nine replicates of each treatment. Regression parameters were estimated using log-linear model with N treatment as a continuous predictor, i.e. Species richness response = Intercept + Slope × Ln (N + 1).

Species was 1.84 g N m\(^{-2}\) yr\(^{-1}\), with a 95% CI of 0.59–3.92 g N m\(^{-2}\) yr\(^{-1}\). The negative effect of N addition on SR was greater in the wet year (2008) than dry year (2009), particularly at moderate and high addition rates (5.25 and 10.5 g N m\(^{-2}\) yr\(^{-1}\)). For both common and rare species, the reduction in SR tended to increase with experimental duration across different N addition rates from 5.25 to 28.0 g N m\(^{-2}\) yr\(^{-1}\).

**Above-ground biomass, intra- and interannual variability and drought resistance**

AGB was significantly affected by N treatment, experimental duration and their interactions (table 1). Although the critical N addition rate for AGB varied substantially over time (year), it was generally lower than 5.25 g N m\(^{-2}\) yr\(^{-1}\) (see electronic supplementary material, figure S1). Throughout the five years, the AGB response showed a log-linear function of N addition rate (see the electronic supplementary material, figure S1). The slope of the biomass–N relationship tended to increase with June–July precipitation (R\(^{2}\) = 0.63, p = 0.0687, N = 5; data not shown). It was significantly greater in normal and wet years (e.g. 2006 and 2008) than in dry years (e.g. 2005, 2007 and 2009; p < 0.01; see electronic supplementary material, figure S1). For example, AGB increased by 87 per cent in 2008 (a wet year; June–July precipitation = 170.7 mm) and only by 32 per cent in 2009 (an extremely dry year, June–July precipitation = 93.7 mm) at the additional rate of 10.5 g N m\(^{-2}\) yr\(^{-1}\), compared with control plots (see the electronic supplementary material, figure S1).

The coefficients of intra-annual variation in AGB did not show significant response to N addition (figure 3a). The coefficients of inter-annual variation in AGB at first increased with N addition rate, peaked approximately at the addition rate of 17.5 g N m\(^{-2}\) yr\(^{-1}\), and then declined at the highest N addition rate (figure 3b). For a given treatment, the magnitude of inter-annual variation in AGB was generally greater than the intra-annual variation (variation among replicates). The drought resistance declined with increasing N addition rate (figure 3c).
soil acidification experiment, in contrast, no significant reduction in SR was observed until the soil pH exceeded 1.2 by the third year (2011) (figure 4a). In the N addition experiment, SR response was negatively correlated with AGB response, which explained 81 per cent of the variance in richness (figure 4b). Greater reduction in SR occurred with larger biomass increment, corresponding to high levels of N addition rates (i.e. 10.5 to 28.0 g N m\(^{-2}\) yr\(^{-1}\)). Stepwise multiple linear regressions, using the \(D_{\text{soil pH}}\) and biomass increase as independent variables, further revealed that biomass increase had much greater partial \(R^2\) (partial \(R^2 = 0.39, p < 0.0001\)) than that of the \(D_{\text{soil pH}}\) (partial \(R^2 = 0.08, p = 0.0082\)).

**Figure 3.** Effects of N addition on intra- and inter-annual variation in above-ground biomass (AGB) and drought resistance (error bars denote s.e.m.). (a) Intra-annual CV\(_{\text{AGB}}\); (b) Inter-annual CV\(_{\text{AGB}}\); (c) AGB\(_{2009}/\text{AGB}_{2006}\). Intra-annual CV\(_{\text{AGB}}\) for each treatment was the average of five years (2005–2009). Inter-annual CV\(_{\text{AGB}}\) and AGB\(_{2009}/\text{AGB}_{2006}\) for each treatment were the average of nine replicates.

**Figure 4.** Relationships of species richness response with changes in (a) soil pH and (b) above-ground biomass (AGB) (error bars denote s.e.m.). In the N addition experiment, species richness and AGB response ratios were the average of nine replicates of each treatment. In the soil acidification experiment, species richness response ratio was the average of five replicates of each treatment in 2011.

**(e) Plant functional traits and trait plasticity**

Among seven common species, which together accounted for 86 per cent of the total community AGB in control plots, the relative AGB of three species significantly increased with N while that of the other four species declined (see electronic supplementary material table, S3). Multi-trait comparison showed that positive and negative response species differed substantially in nine out of 19 plant functional traits, including whole plant, leaf, root, reproductive and life-history traits (see electronic supplementary material, table S3). Species showing positive response to N exhibited greater mass-based leaf N content, stem:leaf biomass ratio, net photosynthetic rate, reproductive allocation and specific root length of the second- and third-order roots but lower leaf C: N and root:shoot ratio than those showing negative response to N (see electronic supplementary material, table S3). For the positive response species, plasticity of both mass- and area-based leaf N contents to medium rate of N addition (5.25 g N m\(^{-2}\) yr\(^{-1}\)) were significantly lower than those of negative response species, with other traits showing little plasticity (see electronic supplementary material, table S3). In addition, our results illustrated that rare species, in general, showed lower photosynthetic rate and stem:leaf ratio but higher...
root: shoot ratio, compared with common species (data not shown).

4. DISCUSSION
(a) Patterns of species richness response to chronic N enrichment and critical threshold
Our results have shown that patterns of SR response differed substantially among N treatments, between the common and rare species, and through time. These patterns suggest the following four conclusions.

First, plant diversity was highly sensitive to chronic N enrichment in the semiarid steppe, and species loss occurred even at very low rates of N input. Although we did observe a critical threshold for N-induced species loss within the range of N addition rates tested in this study, the predicted \(N_{\text{crit}}\) was 0.85 g N m\(^{-2}\) yr\(^{-1}\). This is only partially showed by our results that SR decreased by 15 per cent relative to control even at the addition rate of 1.75 g N m\(^{-2}\) yr\(^{-1}\), below which we lack data to estimate the actual \(N_{\text{crit}}\) for our system. However, the predicted \(N_{\text{crit}}\) is within the range of \(N_{\text{crit}}\) (0.5–2.0 g N m\(^{-2}\) yr\(^{-1}\)) reported from the North American and European grasslands [4,32].

Second, the rare species, which accounted for up to 80 per cent of the total plant species numbers, were more sensitive to chronic N enrichment than common species. The average numbers of rare species reduced by 47 per cent relative to control at the rate of 1.75 g N m\(^{-2}\) yr\(^{-1}\), implying that the actual \(N_{\text{crit}}\) for rare must be lower than our lowest addition rate. This is most probable because of the unique geographical and climatic conditions of the Inner Mongolian steppe, where species have evolved strategies of conservative resource use in water limited and infertile conditions [35,45]. The Inner Mongolian steppe historically received little N deposition (less than 0.05 g N m\(^{-2}\) yr\(^{-1}\)), and the current level of N deposition is still very low (less than 0.3 g N m\(^{-2}\) yr\(^{-1}\)); although it is projected to have N deposition 0.5–1.0 g N m\(^{-2}\) yr\(^{-1}\) in 2030 [4]. Hence, it has not yet experienced a rapid loss of plant species induced by chronic N input. The North American and European grasslands, in contrast, have received high levels of anthropogenic N deposition for several decades, and the loss of highly sensitive rare species has already occurred [4,32,46]. Our findings highlight the impacts of chronic low-level N enrichment on highly sensitive rare species and ecosystem functioning.

Third, the responses of SR and AGB to N enrichment tended to saturate at addition rates more than or equal to 10.5 g N m\(^{-2}\) yr\(^{-1}\), beyond which little additional increase in biomass and/or reduction in plant diversity were observed. This is consistent with our previous findings from the first 4-year results of the same experiment [8], and it is also supported by long-term data of extractable soil NH\(_4\)-N and NO\(_3\)-N concentrations (Y. F. B. Bai 2010, unpublished data).

Fourth, the negative impacts of N enrichment on SR seemed to increase with N addition duration. Our results showed that SR response ratio declined with increasing experimental duration, particularly at moderate (5.25 g N m\(^{-2}\) yr\(^{-1}\)) and high addition rates (10–28 g N m\(^{-2}\) yr\(^{-1}\)). This finding is further corroborated by our previous study using the first 4-year data from the same experiment [8].

(b) Water-mediated N effects
Our results demonstrate that the June–July precipitation was an important predictor of AGB and SR responses to N. Greater responses of AGB to N addition were found in wet years, with high June–July precipitation (e.g. 2008). The reduction in SR relative to control also tended to be greater in wet years than dry years at addition rates of 1.75–10.5 g N m\(^{-2}\) yr\(^{-1}\). These results suggest two conclusions: first, above-ground net primary production is co-limited by water and N availability. Although we did not find strong evidence for a critical precipitation level, below which water availability was seriously limiting and AGB exhibited little response to N, our results showed that AGB response saturated at addition rates more than or equal to 5.25 g N m\(^{-2}\) yr\(^{-1}\) in 2009 (an extremely dry year), indicating that primary production was more limited by water than N. Second, the seasonal distribution of precipitation, such as June–July precipitation, is more important than total annual precipitation in controlling community and species responses to N. This may be because N was added in early July each year, and temperature usually reached its annual peak in July. If the precipitation was also high in June and July, all of these key factors could be synchronized in a manner stimulating plant growth [34,37]. Therefore, our results are consistent with the pulse-reserve/threshold hypothesis that plant growth and primary production in drylands are driven by the complex interactions among key abiotic and biotic factors [36].

(c) Mechanisms underlying N-induced species loss
Our results demonstrated that rare species were highly sensitive to N enrichment, with low initial abundance (less than 1%) and low critical threshold but high risk of species loss. The loss of rare species occurred continuously throughout the experimental period from 2000 to 2009 (also see Bai et al. [8]) and across all N addition rates. Our findings support the general projections of the random loss hypothesis which posits loss of rare species with N enrichment is mostly owing to their low initial abundance. However, our results showed that rare species generally exhibited the lower photosynthetic rate and stem : leaf ratio but higher root : shoot ratio, compared with common species. This indicates that both the low initial abundance and low above-ground competitive ability may be attributable to the loss of rare species in our system.

For common species, shifts in species dominance and composition were mainly caused by functionally based mechanisms. The experimental evidence supports our original hypotheses that chronic N enrichment increased the relative importance of above-ground competition over below-ground competition. We found that shifts in species dominance favoured acquisitive versus conservative resource-use strategies. Species with acquisitive resource-use strategies showed high leaf N content, net photosynthetic rate and stem : leaf ratio.
but low root: shoot ratio, corresponding to high above-ground/light competitive ability. These species, such as *A. amaranthoides*, *C. glaucum* and *L. chinensis*, exhibited competitive advantage over others when N limitation was released following N enrichment. In contrast, species with conservative resource-use strategies were characterized by high leaf C:N, specific leaf area and root: shoot ratio but low leaf N content and net photo-synthetic rate, corresponding to high below-ground competitive ability. Hence, species that dominated at original community, such as *S. grandis*, *A. cristatum* and *A. sibiricum*, were potentially outcompeted by those with high above-ground competitive ability following N enrichment. The trait trade-offs of plants observed in our experiment reflect the nature of fundamental trade-offs between productivity and persistence among coexisting species [47].

It has been proposed that recruitment limitation is predominant in underpinning the chronic N enrichment-induced loss of plant species [4,22]. Our findings illustrated that species with acquisitive resource-use strategies exhibited substantially higher reproductive output than those with conservative resource-use strategies. This indicates that, for resource conservative species, recruitment limitation was intensified owing to low reproductive output and competitive disadvantage in above-ground competition. Our field observations further revealed that the seedling density of these resource conservative species was very low following 10 years of N enrichment. For species with acquisitive resource-use strategies, however, greater seedling density was observed (Z. Lan, X. Bai and Y. Bai 2011, unpublished data). Thus, we conclude that chronic N enrichment increased recruitment limitation of the resource conservative species.

Our results, in general, support the projections of the soil acidification hypothesis, but we further found that the effect of soil pH reduction on species loss was relatively minor in magnitude compared with the direct effect of chronic N input. Multiple linear regressions revealed that the proportion of variance in SR explained by biomass increment was much greater (partial $R^2 = 0.39$) than the soil pH reduction (partial $R^2 = 0.08$) in the N addition experiment. Thus, the chronic N enrichment-induced biomass increment is likely to be a major driver of N-induced plant species loss. Our findings were different from those of previous studies in the European grasslands regarding the relative importance of N availability versus soil acidification [4,24,25]. This may be attributed to high initial soil pH, high CEC and low mean annual precipitation in the semiarid Inner Mongolia grassland, which together suggest that the soils of our system are less sensitive to acidification and nutrient cation loss than those of acidic soils in the European grasslands following N addition [4,9].

### (d) Implications of ecosystem functioning and services under global change

Our findings have shown that chronic N enrichment substantially reduced plant species diversity, led to the loss of species with resource conserving strategies and shifted species dominance. These changes in plant diversity and species composition affected ecosystem functioning and provisioning of services in different ways. First, stability of primary production was reduced considerably. The decreased stability is mainly attributed to the increased inter-annual variations in AGB with N addition. Second, ecosystem drought resistance declined with increasing N addition rate. This is probably caused by the reduction in RA of perennial bunchgrasses (e.g. *S. grandis*, *A. cristatum* and *A. sibiricum*) and increased dominance of N demanding annuals (i.e. *C. glaucum* and *A. amaranthoides*). Third, the replacement of perennial bunchgrasses by annuals may also decrease below-ground biomass allocation and reduce non-growing vegetation cover. This could diminish snow-holding capacity, increase soil erosion owing to the strong fall and winter winds and reduce soil carbon sequestration potential [48].

Our findings have important implications for better understanding the effects of global change on ecosystem services of the Inner Mongolia grassland. It has been projected that future climatic change will increase the extreme droughts and intense rainfall events [16,49]. The projected increase in N deposition and change in precipitation regimes may act together to accelerate the loss of rare species, shift species dominance and composition, elevate water limitation, and consequently diminish ecosystem stability and services [4,14,16,37].

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